

## Metal Ions in Fur as a Bio-monitor of the Systemic Accumulation in Albino Rat

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**Abstract:** The study carried out a periodic quantification of the amounts of metal ions accumulated in the blood of Wistar albino rat pre- and post-operative the implantation of stainless steel (SS) arch bar used for maxillomandibular fixation and compares these with the amounts accumulated in the fur used as a bio-monitor. The concentrations of the corrosion products increased from the 3 to 6 weeks post-operative periods in the blood and fur. The total metal ions accumulated in the fur and blood of Wistar albino rat *in vivo* follows the ranking Ni > Fe > Mn > Co > Cr. Ni shows a high affinity for the fur and blood. The concentrations of Co, Mn and Cr ions in the fur post-operative is indicative of the levels of these metal ions in the blood.

**Key words:** Arch bar, corrosion product, blood, fur and bio-monitor

### INTRODUCTION

The systemic effects of the particulate debris of metallic implants will depend upon the origin, the volume, the size and the shape of the debris, as well as on its chemical composition, physical behaviour at the cellular and extracellular levels, the body's response pattern, possible cellular activation mechanisms, local and/or systemic actions and the time course of the process (Willert *et al.*, 1993; Allen *et al.*, 1997).

Like other substances transported in the blood or the lymph, particles and particle breakdown products may be eliminated through the body's excretory mechanisms. Levels of released metal ions may be detected and determined in body fluids such as serum or plasma, urine and sweat (Howie *et al.*, 1996; Merrit and Brown, 1996). Urine and serum metal concentrations, however, are dependent on the excretory rate of the metal ions which is a highly individualised parameter and species specific. Hair has the advantage of long term memory; a three-inch strand of human hair will give a six month history of the body chemistry (Wein-Schwartz and Oderda, 2000). Clinical research indicates that the hair levels of specific elements, particularly toxic elements such as cadmium, mercury, lead and arsenic, correlate with pathological disorders (Sharma and Kumar, 2004); the levels of the elements in the hair provide a superior indication of body stores opposed to blood or urine specimens (Wein-Schwartz and Oderda, 2000; Mehra and Juneja, 2005).

The concentration of any corrosion product at local or systemic sites will depend on the rates of diffusion, applicable chemical reactions, such as precipitation or complexing, excretion processes, uptake rates and affinity toward remote organs (Van-Noor, 1987). The observation that metal ions can be detected in remote tissues including

hair, urine and peripheral blood (Katharine and Stanley, 1985) indicates that much of the metal released from the implant is transported away from the site (Simpson *et al.*, 1973). The concern about the release and distribution of metal degradation products is justified by the known potential toxicities of Ti, Al, V, Co, Cr and Ni (the elements used in modern orthopaedic alloys) (Black, 1988; Wapner, 1991).

Albert *et al.* (1962) embedded Vitallium, 316 stainless steel, Incoloy, A-286 stainless steel, aluminium 2024-T3, titanium and zirconium in the skeletal muscles of albino rabbits - one alloy to any one animal. Spectrographic analyses of the trace-metal concentration of the surrounding muscle, spleen, lung, liver, kidney and control muscle showed that the spleen was found to be the most active site of trace-ion storage, the other organs were tending to get rid of an early increase in concentration. Cobalt and nickel were the most active ions in terms of their presence in these organs.

This study reports the comparative evaluation of the periodic metal ions concentrations in the fur of albino rat pre- and post-operative maxillomandibular fixation of stainless steel arch bar to the levels in blood. The role of corrosion products in adverse local and systemic tissue reactions is of clinical pertinence and needs to be thoroughly assessed with the use of hair as a bio-monitor of the post-operative levels of metal ions in the distant organs.

### MATERIALS AND METHODS

Sixteen female albino rats of Wistar strain (181 to 192 g body weight) of age eight weeks were used for the study. The rats were obtained in August, 2008 from the animal house of the Faculty of Pharmacy, Ahmadu Bello

University, Zaria-Nigeria were fed commercial rat pellets (Bendel Feeds and Flour Mill, Ewu, Nigeria) and tap water *ad lib*. Six rats marked A1 to A6 were used for anaesthesia experimentation, in order to determine the quantity of the preanaesthetic (to weaken) and the anaesthetic (to knock off) required for minimum recovery time of the animal after implantation. The experimental rats were marked B1 to B10 and housed in a separate cage.

Subcutaneous (under skin) injection of 0.2 mL chlorpromazine (CLP) - a preanaesthetic-, and 0.3 mL Ketamine (KTM) - an anaesthetic - 15 minutes after, was administered to rats A1 and A2; 0.2 mL CLP and 0.2 mL KTM 15 minutes after, to rats A3 and A4; and 0.2 mL CLP and 0.1 mL KTM 15 minutes after, to rats A5 and A6.

Each of the experimental rats (*Rattus norvegicus*) was anaesthetised with 0.2 mL CLP and 0.1 mL KTM. After which a  $0.6 \pm 0.05$  cm length, as-received stainless steel (SS) arch bar of average weight  $75.05 \pm 0.90$  mg (Unitek, Monrovia, California, USA) was implanted in the lower jaw of the animal and ligated to immobilise with about 3.0 cm length chromic catgut as shown in Plate I. The surgical implantation was carried out at the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria.

The fur of the experimental rats was collected pre-operative, three and six weeks post-operative with a new pair of scissors. In order to determine the metal ions in the blood the rats were bled at the tails 24 hours before the implantation and 3 and 6 weeks post-operative, and a 0.5 mL blood sample was collected with a 2 mL syringe for each time point.

The fur collected pre- and post-operative from the rats was cleaned with doubly distilled water to remove dust particles. The samples were then dried in an oven at 50°C for 6 hours. To each of the samples 3 mL of concentrated HNO<sub>3</sub> and 10 mL of concentrated HCl solution was added on a hot plate at 90°C. The digest was then filtered through a Whatman no. 1 filter paper and made up to 25 mL mark in a volumetric flask with doubly distilled water. The concentrations of nickel, cobalt, manganese, chromium and iron ions in the fur were analysed using atomic absorption spectroscopy (Jones *et al.*, 1987) putting into consideration the mean dry mass of the samples. The blood samples were also digested using the same procedure and metal analysis was then carried out on the 25 mL digest. The six weeks implantation period simulates the healing period after maxillomandibular surgery. All animals received humane care according to the European Convention on Animal Care.

## RESULTS AND DISCUSSION

The results for the average concentrations of accumulated metal ions in the fur and blood with implantation time for n = 10 determinations  $\pm$  the standard deviation are presented in Fig. 1 and 2. The quality

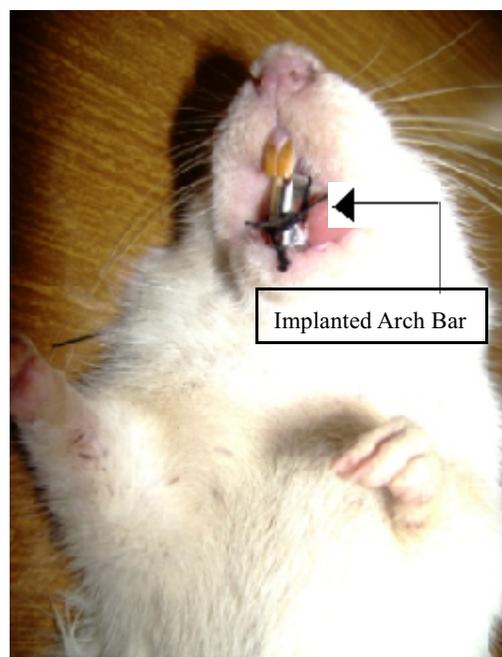


Plate I: Maxillomandibular Fixation of Stainless Steel Arch Bar into an Albino Rat

assurance for the analyses was conducted through the spiking method, to evaluate the sample digestion process and effectiveness of the AAS machine. A chemical analysis protocol through multi-element standard solution (MESS) was applied as follows: 10 mL of pre-digested sample was measured into a 250 mL beaker and spiked with 10 mL of MESS at level of 0.5 mg/L Co, Ni, Fe, Mn and Cr. To this 5 mL of concentrated HNO<sub>3</sub> and 10 mL of HCl was added and the mixture digested. The mean % recovery for the analyses ranged from  $81.9 \pm 0.15$  to  $94.9 \pm 0.40$  mg/kg.

The data analyses was processed using SAS (statistical analysis software). Duncan's Post-hoc multiple range test (DMRT) was used to assess the statistical difference in the mean values of the various metal ions accumulated by the blood and the fur ( $P < 0.05$ ). The study further considered the possibility of establishing a statistical relationship between the metal ions accumulated in the fur and that in blood pre- and post-operative MMF of SS arch bar in the rats.

Determination of the doses of chlorpromazine, CLP, and Ketamine, KTM, needed for minimum recovery of the rats after the implantation of SS arch bar as presented in Table 1 showed that administration of 0.2 mL of CLP and 0.1 mL of KTM to the animals resulted to recovery after 7 hours.

As shown in Fig. 1, following the implantation of SS arch bar into the rats mean concentration of Co ions in blood increased from the 3 weeks to 6 weeks implantation period ( $0.0297 \pm 0.0062$  to  $0.0387 \pm 0.0090$  mg/kg). A sharp increase in level of Fe ions in blood was recorded in the 3 weeks to 6 weeks post-operative periods. The amount of Mn ions in blood was increased from the

Table 1: Anaesthesia Test on Albino Rats

Rat Code	Average Body Weight (g)	CLP (mL)	KTM (mL)	Average Recovery Time (h)
A1 and A2	184	0.2	0.3	24
A3 and A4	189	0.2	0.2	16
A5 and A6	182	0.2	0.1	7

CLP – Chlorpromazine; KTM - Ketamine pre-opert - pre-operative; post-opert - post-operative

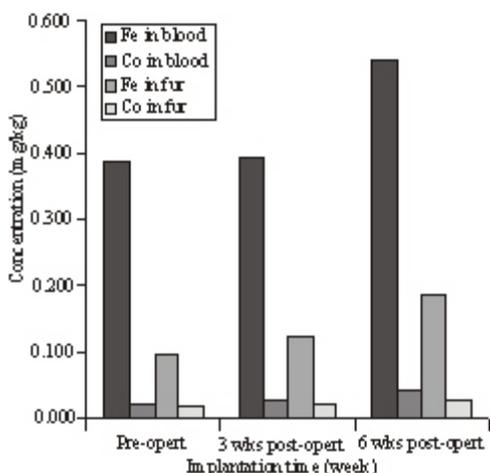


Fig. 1: Concentration of Fe and Co ions in blood and fur with time of implantation

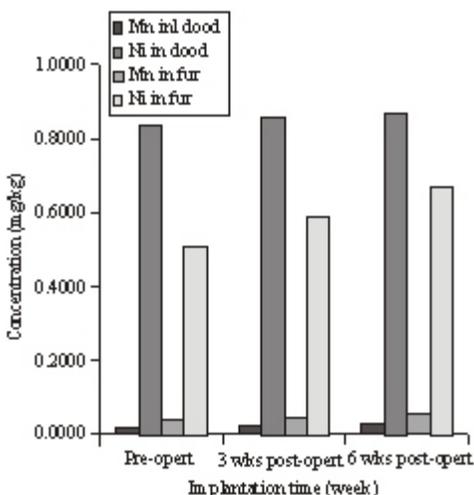


Fig. 2: Concentration of Mn and Ni ions in blood and fur with time of implantation

pre-operative value of  $(0.0215 \pm 0.0062)$  to  $(0.0295 \pm 0.0031)$  in the 6 weeks post-operative sample (Fig. 2). From Fig. 2 the mean concentration of Ni ions in blood was  $0.8610 \pm 0.0078$  mg/kg in the 3 weeks post-operative sample and closely followed by  $0.8764 \pm 0.0050$  mg/kg in the 6 weeks sample.

The mean concentrations of the corrosion products of SS arch bar in the fur increased from the 3 to 6 weeks implantation periods; the amount of Fe ions in the 6 weeks post-operative sample doubles the value in the pre-

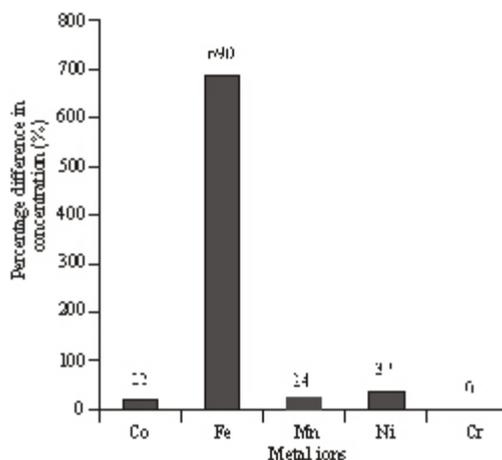


Fig. 3: Percentage difference between Mean Metal ions concentration of Fur and Blood of Albino Rat (%)

operative (Fig. 1). However, Ni ions concentration in fur was highest in folds of five, twenty and ten respectively compared to Fe, Co, Mn ions. The level of Cr ions was below detection limit in the blood and fur samples of the animal model.

From the results pre- and post-operative levels of metal ions in blood was highest for Ni ions ( $0.8565 \pm 0.0203$  and  $0.8764 \pm 0.0050$  mg/kg for pre- and 6 weeks post-operative respectively) and least for Cr ions. The pre- and post-operative metal ions concentration in fur was also highest for Ni ions ( $0.5122 \pm 0.0233$  and  $0.6703 \pm 0.0513$  mg/kg for pre- and 6 weeks post-operative respectively) and least for Cr ions. Further investigation using Duncan’s ANOVA grouping informed that no significant difference existed for mean concentrations of Co and Mn ions (post-operative) in the blood and fur of the animals, a slightly significant difference for Fe ions and a high level of significance for Ni ions ( $P < 0.05$ ). The total metal ions accumulated in the fur and blood of Wistar albino rat *in vivo* follows the ranking Ni > Fe > Mn > Co > Cr. Ni shows a high affinity for the fur and blood. Pearson’s correlation between the total metal ions in the fur and blood (post-operative) gave a correlation coefficient of 0.9019. These results show that a relationship existed between the accumulated metal ions in blood and fur of the rats. And conforms with the findings of Bishara (1993) that new, ‘as received’ orthodontic appliance used for malocclusions release Ni and Cr ions in levels tolerated by the body.

As shown in Fig. 3, the percentage differences between the mean concentrations of the total metal ions in fur and blood was about 20% for Co and Mn ions, Ni about 35% while Fe ions in blood was about seven-fold that in fur. It is considered from this result that the concentrations of Co, Mn and Cr ions in the fur post-operative is indicative of the levels of these metal ions in the blood. This study furnishes added scientific data to the fact that stainless steel arch bar release metal ions into the blood of host and may accumulate in distant organs; the

fur being a bio-monitor for periodic evaluation of the systemic effects on host.

This finding conforms to the assertion of Sharma and Kumar (2004) that the hair levels of some specific elements correlate with pathological disorders. Therefore, Co, Mn and Cr ions concentrations in the hair of the implant's host in the experimental and clinical settings can serve as a bio-indicator of the levels in the systemic reservoir - local and remote organs.

### CONCLUSION

The results further reaffirm that blood and fur are some of the reservoirs for the corrosion products of stainless steel arch bar. The low levels of accumulation of the anticipated major corrosion products of SS arch bar, Ni, Fe and Cr ions in the organs investigated suggest that there is no potential physiological adverse consequence on the host. However, cautious considerations should be taken into account due to elevation in the levels of leached metal ions through this route especially with the use of restorations with consecutively reused arch bars.

The levels of metal ions released from most reports in human studies appear to be in the range handled by the body's systemic compensatory mechanisms, which adjust levels of trace elements.

Currently there is dearth of information with regard to any remote or systemic effects in human case that have had a stainless steel joint replacement procedure (Poech *et al.*, 1996). Notwithstanding, corrosion of metallic implants remains a serious clinical concern, as deleterious corrosion processes have been observed in certain clinical settings. There is reason to believe that attention to variables related to metallurgical processing, surface-processing modalities and appropriate selection of materials can decrease the rate of corrosion and minimise the potential for adverse clinical outcomes.

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